10044967 .052902

FIG. 1

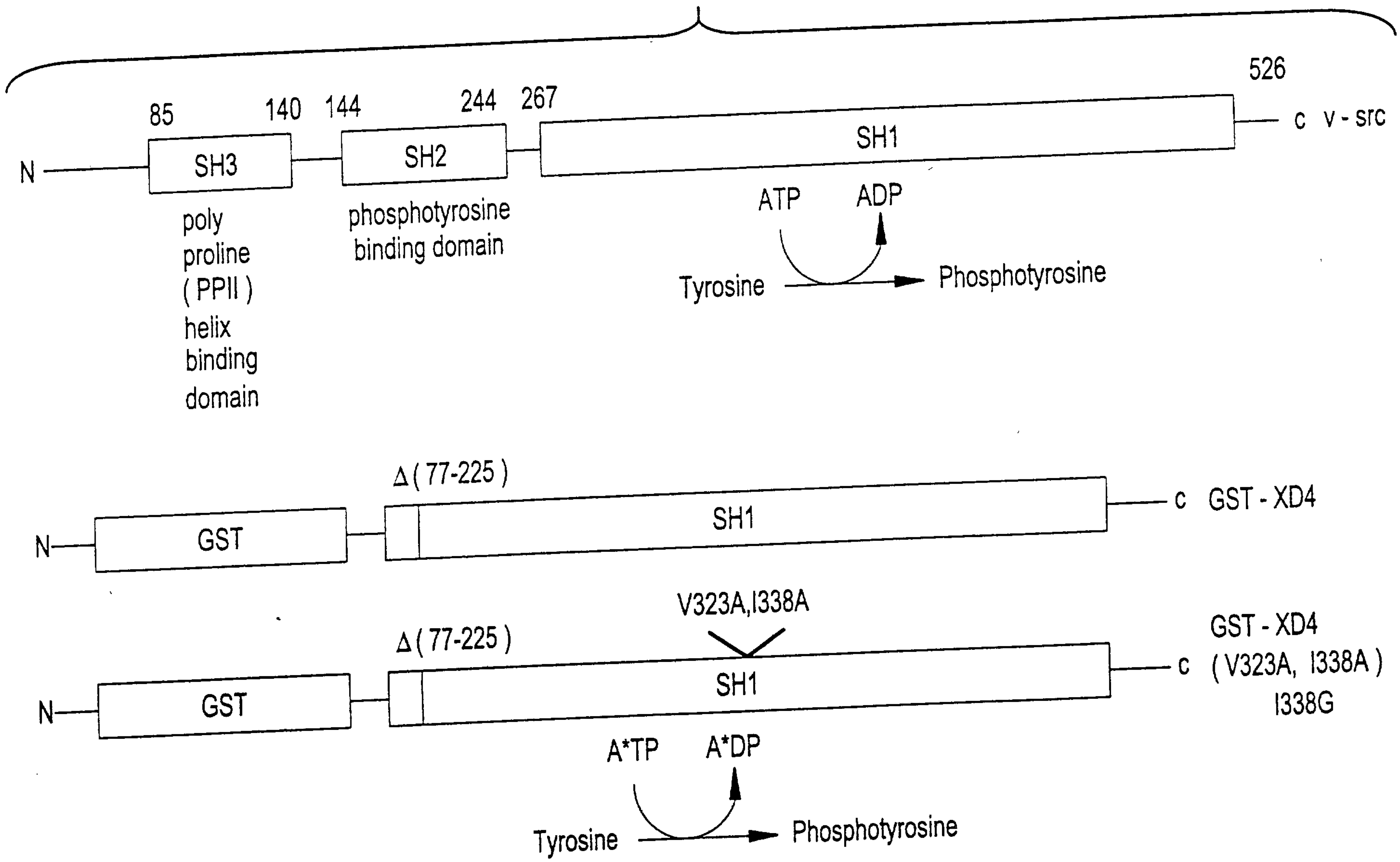
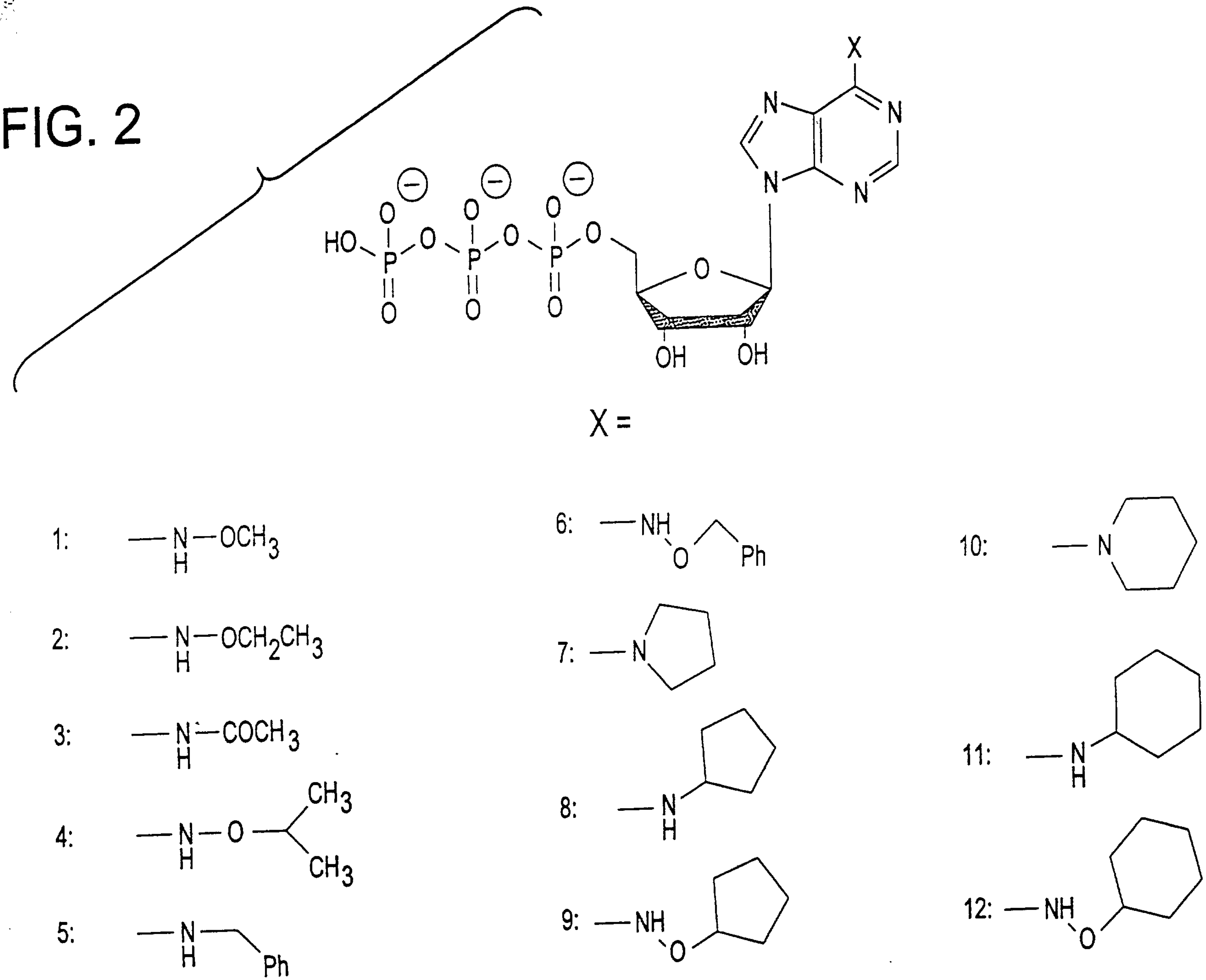


FIG. 2



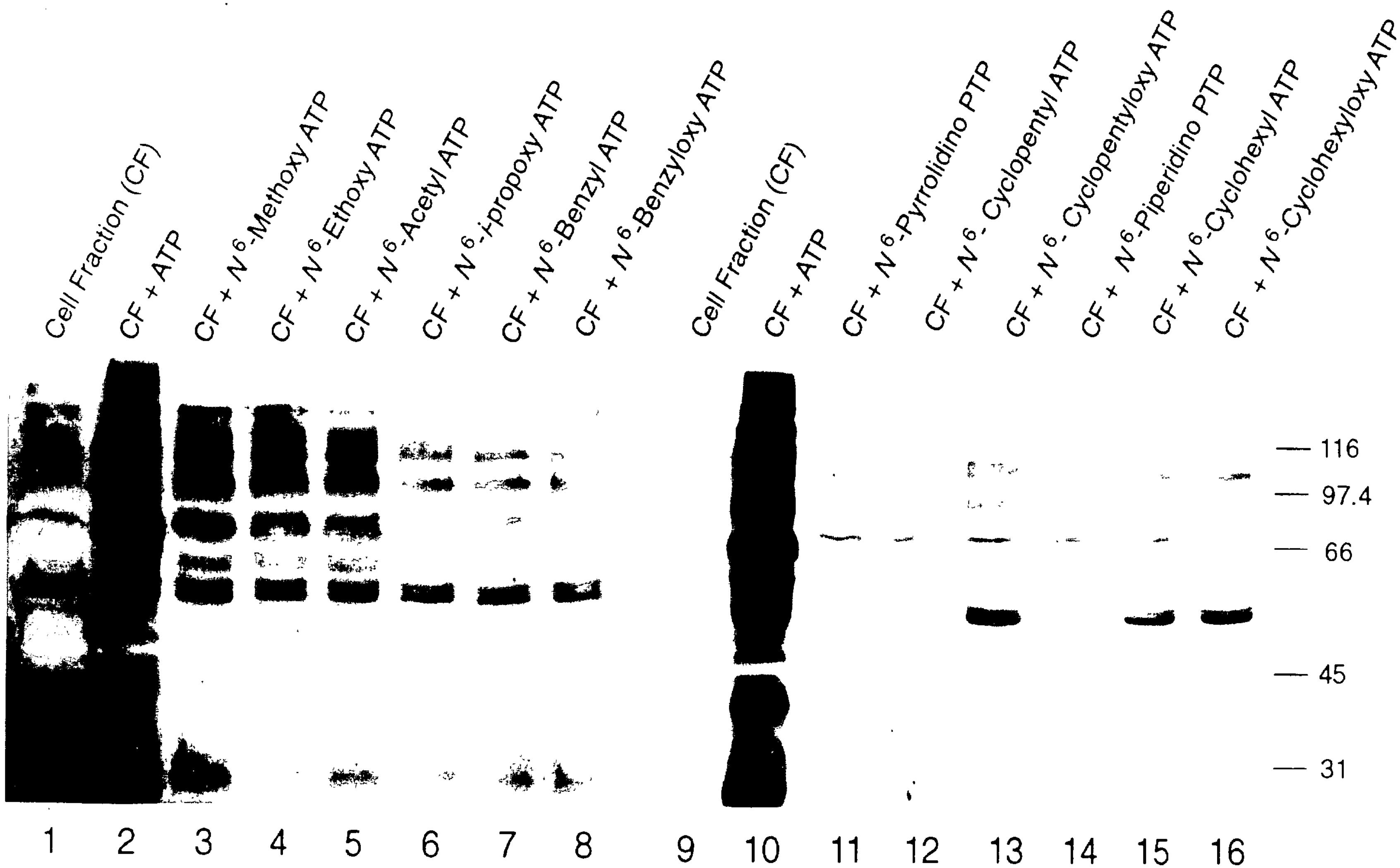

Cell Fraction (CF)
CF + ATP
CF + N^6-Methoxy ATP
CF + N^6-Ethoxy ATP
CF + N^6-Acetyl ATP
CF + N^6-*i*-propoxy ATP
CF + N^6-Benzyl ATP
CF + N^6-Benzyloxy ATP
Cell Fraction (CF)
CF + ATP
CF + N^6-Pyrrolidino PTP
CF + N^6- Cyclopentyl ATP
CF + N^6- Cyclopentyloxy ATP
CF + N^6-Piperidino PTP
CF + N^6-Cyclohexyl ATP
CF + N^6-Cyclohexyloxy ATP
116
97.4
66
45
31
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

FIG.3

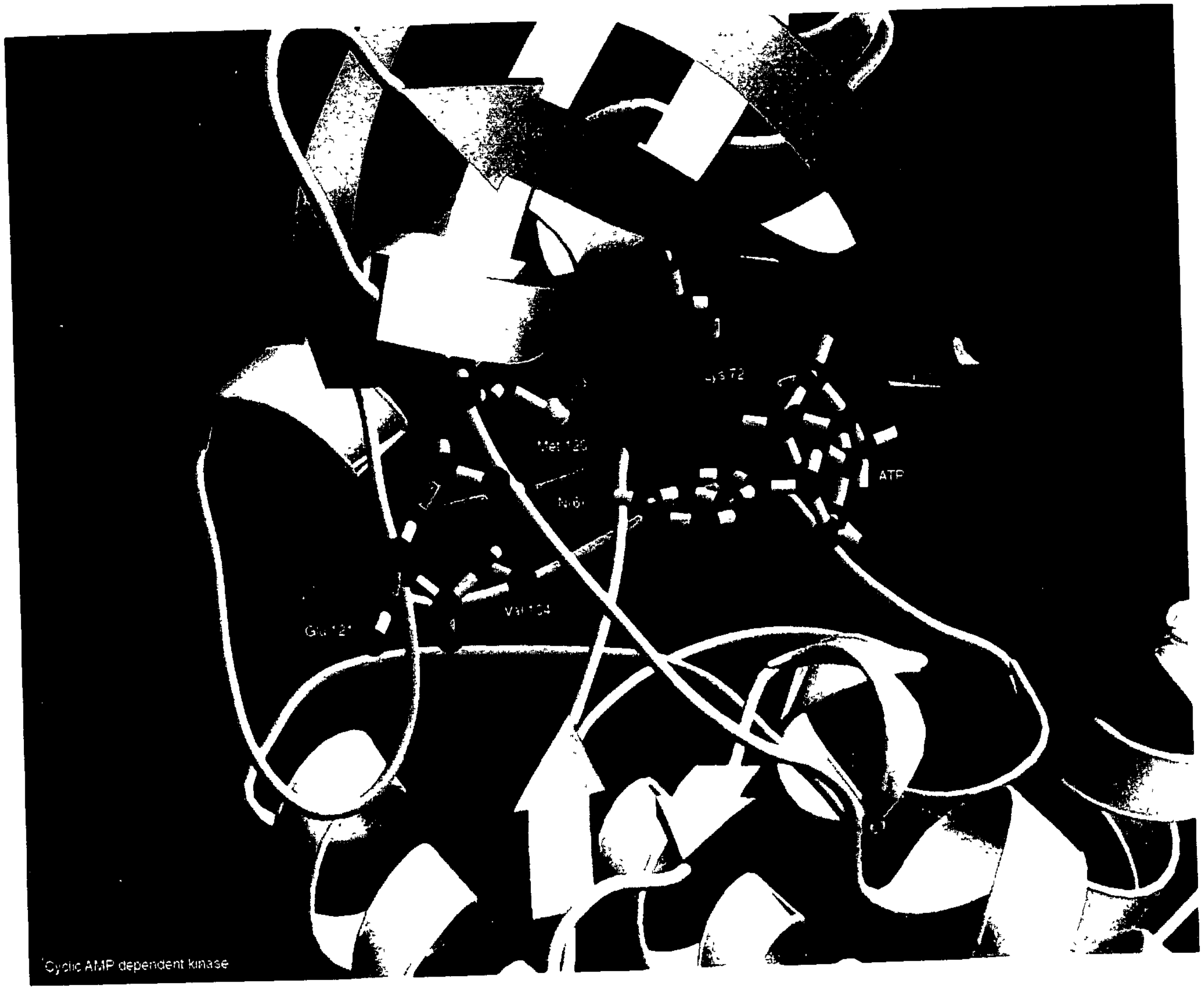


FIG.4

FIG.5A

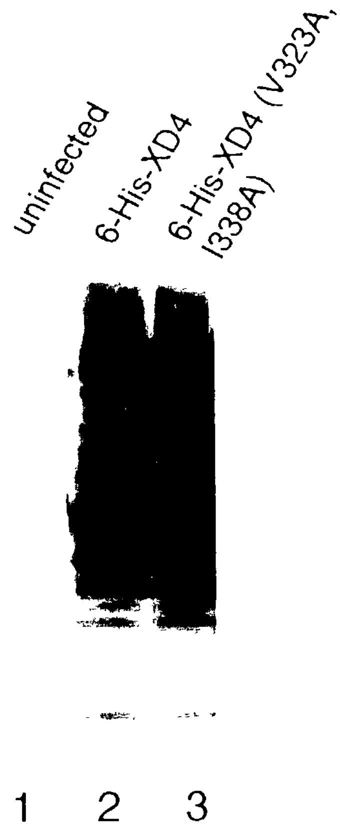


FIG.5B

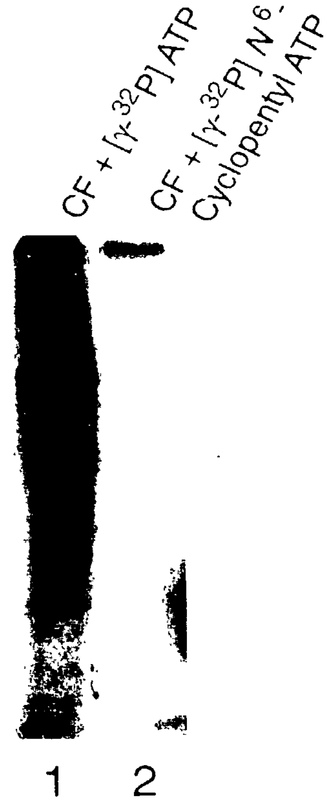


FIG.5C

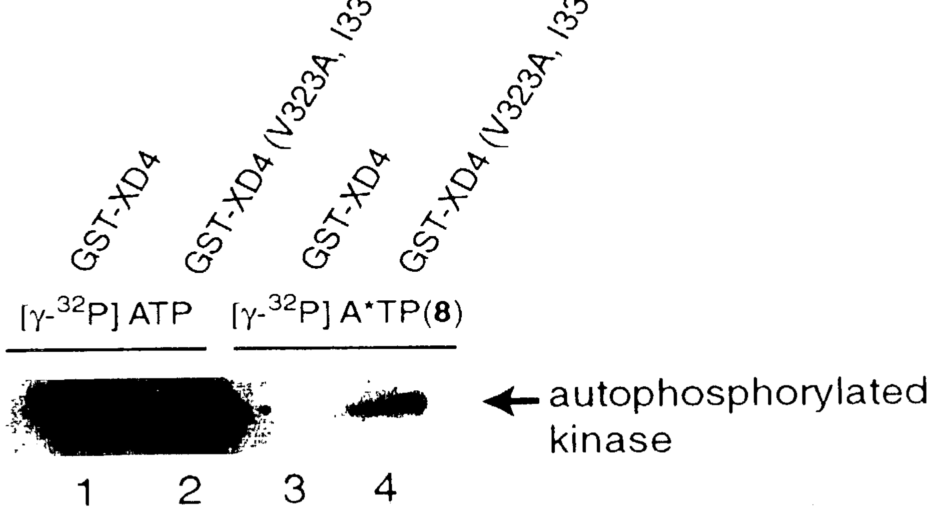


FIG. 6

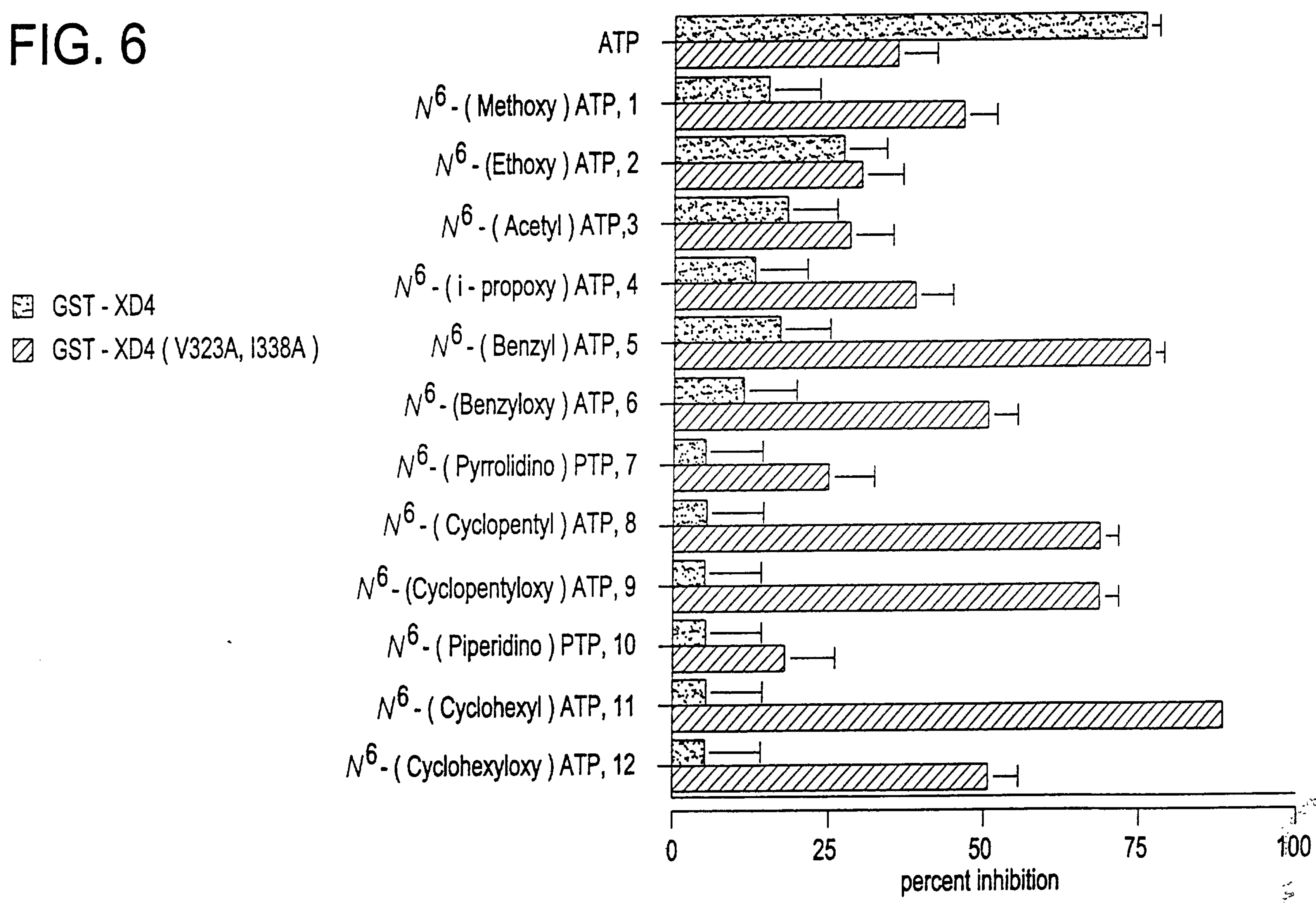

GST - XD4
GST - XD4 (V323A, I338A)
ATP
N6 - (Methoxy) ATP, 1
N6 - (Ethoxy) ATP, 2
N6 - (Acetyl) ATP,3
N6 - (i - propoxy) ATP, 4
N6 - (Benzyl) ATP, 5
N6 - (Benzyloxy) ATP, 6
N6 - (Pyrrolidino) PTP, 7
N6 - (Cyclopentyl) ATP, 8
N6 - (Cyclopentyloxy) ATP, 9
N6 - (Piperidino) PTP, 10
N6 - (Cyclohexyl) ATP, 11
N6 - (Cyclohexyloxy) ATP, 12
0
25
50
75
100
percent inhibition

FIG.7A

[γ-^{32}P] ATP

I338A I338S

FIG.7B

[γ-^{32}P] N^6-cyclopentyl ATP

I338A I338S

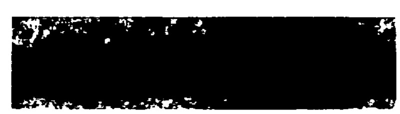
FIG.7C

[γ-^{32}P] ATP

I338A I338G

FIG.7D

[γ-^{32}P] N^6-cyclopentyl ATP



I338A I338G

FIG. 8

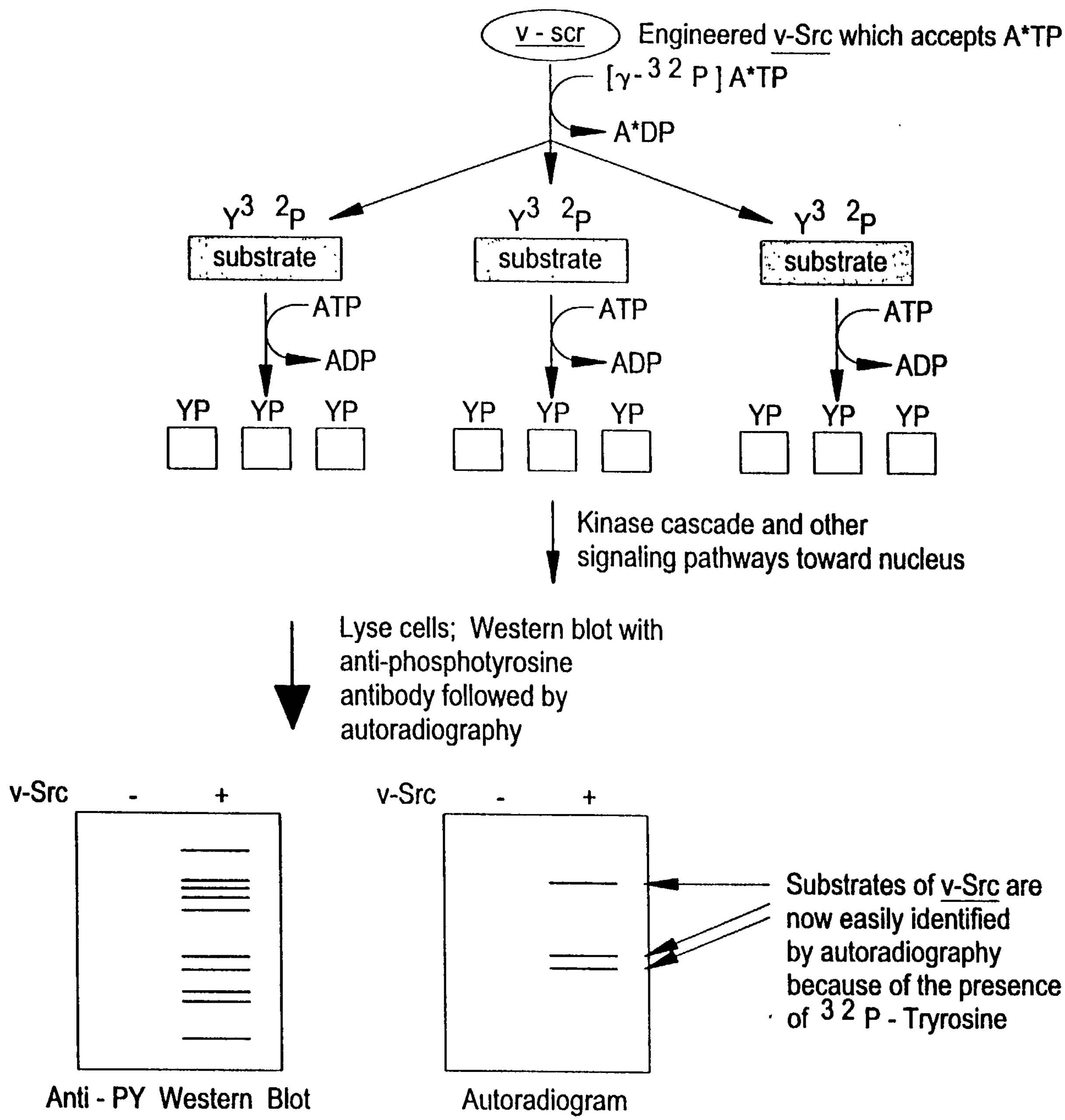
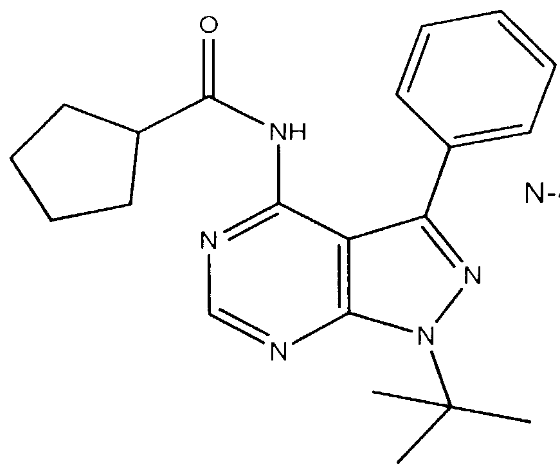

v - scr
Engineered v-Src which accepts A*TP
[γ-32P] A*TP
A*DP
Y32P
substrate
Y32P
substrate
Y32P
substrate
ATP
ADP
ATP
ADP
ATP
ADP
YP YP YP
YP YP YP
YP YP YP
Kinase cascade and other signaling pathways toward nucleus
Lyse cells; Western blot with anti-phosphotyrosine antibody followed by autoradiography
v-Src - +
v-Src - +
Substrates of v-Src are now easily identified by autoradiography because of the presence of 32P - Tyrosine
Anti - PY Western Blot
Autoradiogram

FIG. 11A



N-4 cyclopentoyl PP3

FIG. 11B

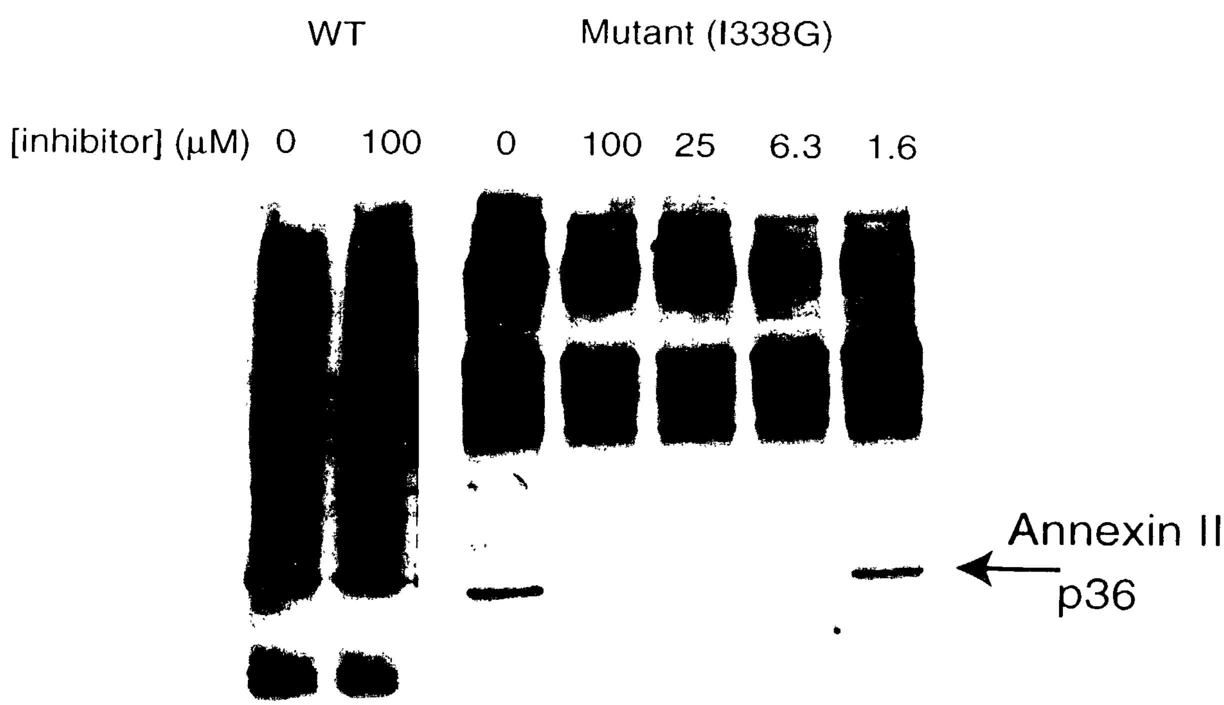
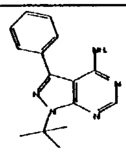
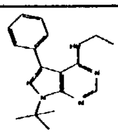
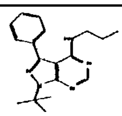
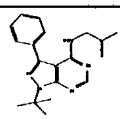
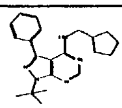
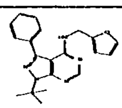
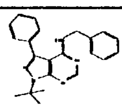
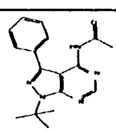
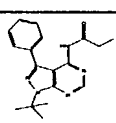


FIG. 12A

IC_{50} (μM)

	Molecule	WT XD4	I338G XD4	WT Fyn	T339G Fyn	WT Abl	T120A Abl
a		35	0.13	0.05			<<10
b			200	>300			
c			300	>300			
d			>300	>300			
e		>300	75	>300	100		>10
f		>300	250	>300	26		>10
g		>300	85	>300	63		>10
h							
i							

10044967.052902

FIG. 12B

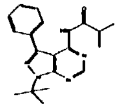
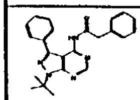
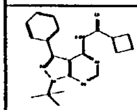
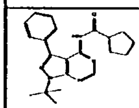
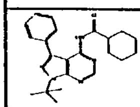
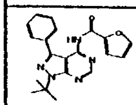
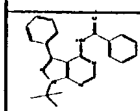
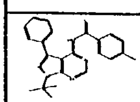
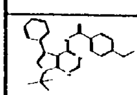
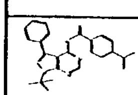
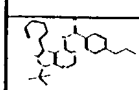
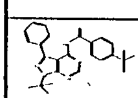
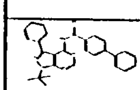
j							
k							
l		>300	12	6.5	5		
m		>300	19	80	9		
n		>300	20	50	5		
o		>300	150	15	19		
p		>300	10	300	11		(10
q		>300	10	300	6		(10
r			1.2				<10
s			0.63				
t			(0.411				1.8
u		>300	0.43	300	0.83	300	(10
v							

FIG. 12C

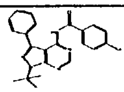
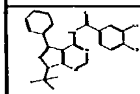
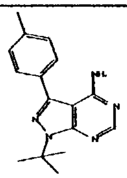
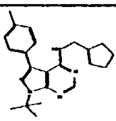
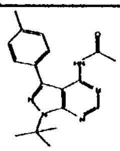
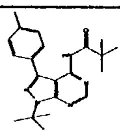
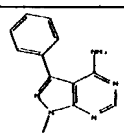
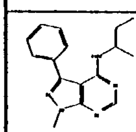
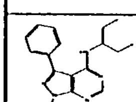
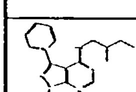
w							
x							>10
y		100	(0.05	0.1			
z			>100	>300			
aa				2			
bb				7			
cc							
dd							
ee							
ff							

FIG. 12D

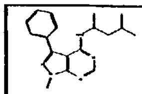
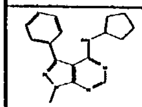
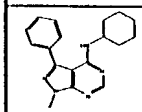
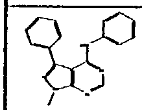
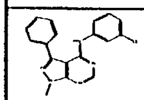
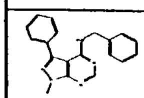
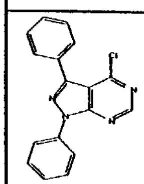
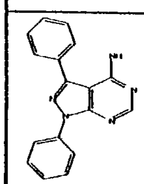
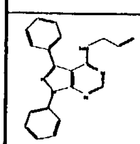
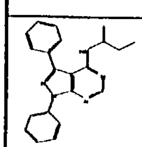
gg							
hh							
ii							
jj							
kk							
ll							
mm							
nn		>1000	0.510	0.4		<<6.5	
oo		>300	>10	>300			
pp		>300	>10	>300			

FIG. 12E

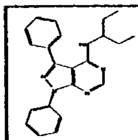
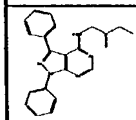
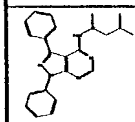
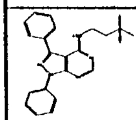
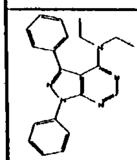
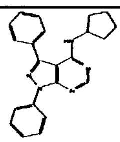
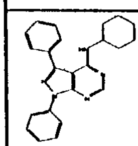
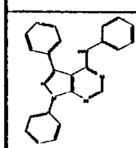
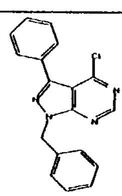
qq		>300	>10	>300			
rr		>300	>10	>300			
ss		>300	>10	>300			
tt		>300	>10	>300			
uu		>300	>10	>300			
vv		>300	>10	>300			
ww		>300	>10	>300			
xx		>300	>10	>300			
yy							

FIG. 12F

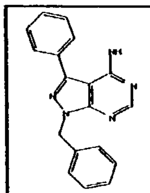
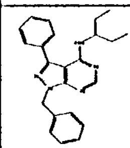
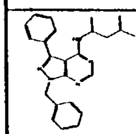
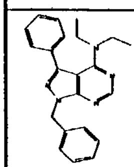
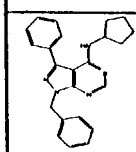
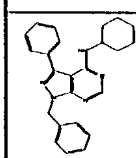
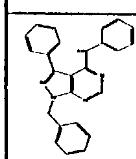
zz		<10	2.5	<<10			
aaa		>300	>10	>300			
bbb		>300	>10	>300			
ccc		>300	>10	>300			
ddd		>300	>10	>300			
eee		>300	>10	>300			
fff		>300	>10	>300			

FIG. 13A

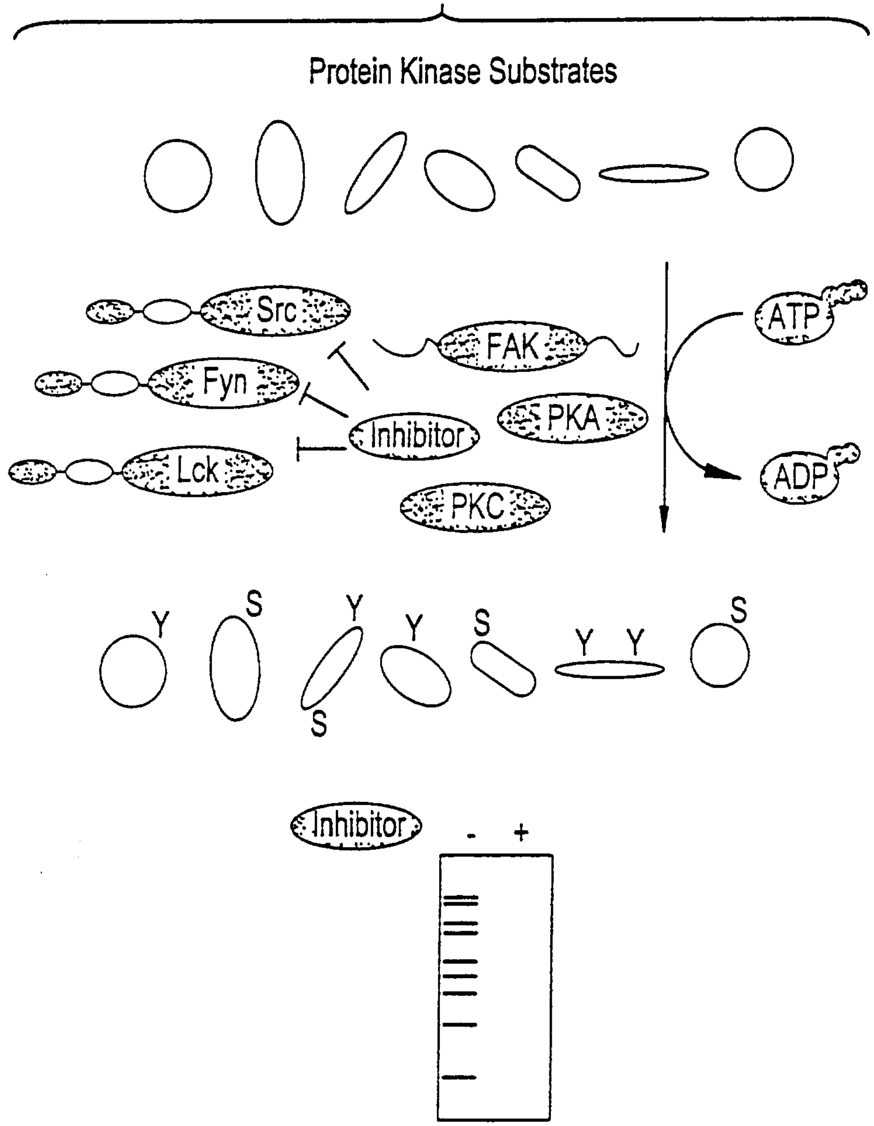


FIG. 13B

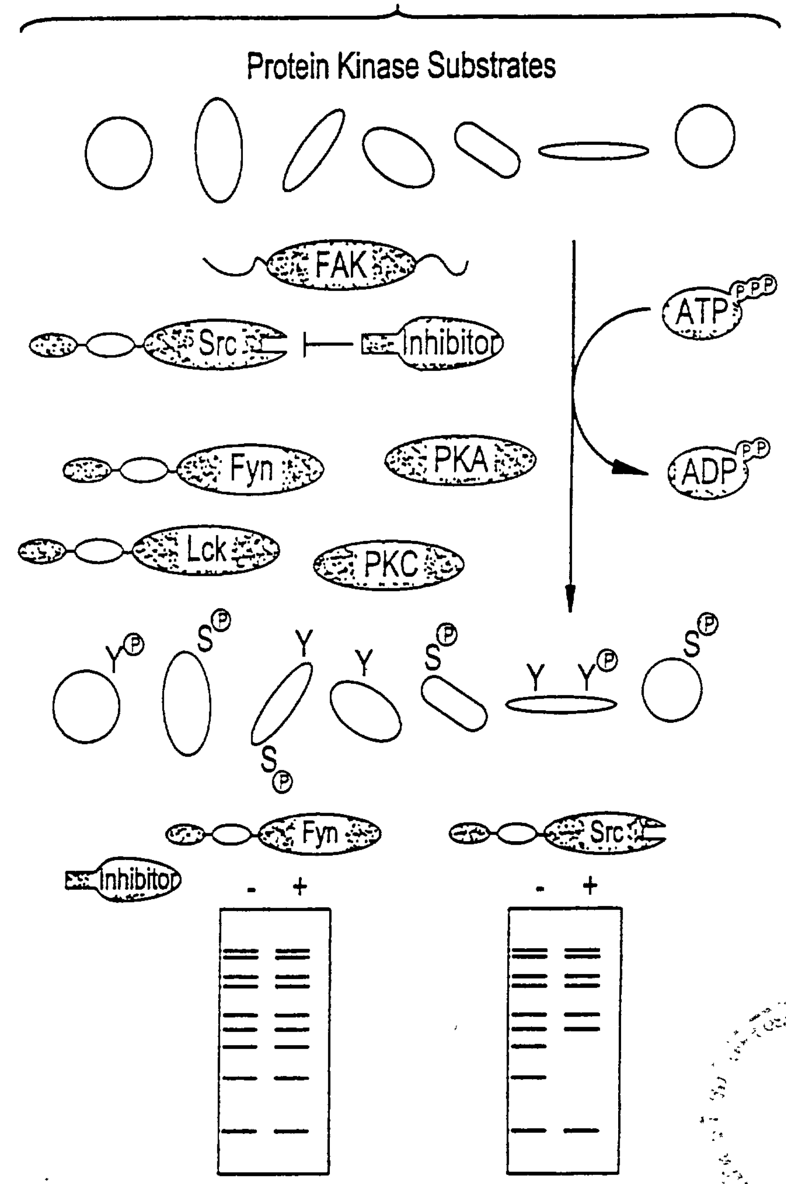
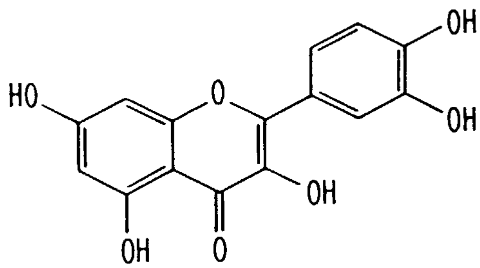
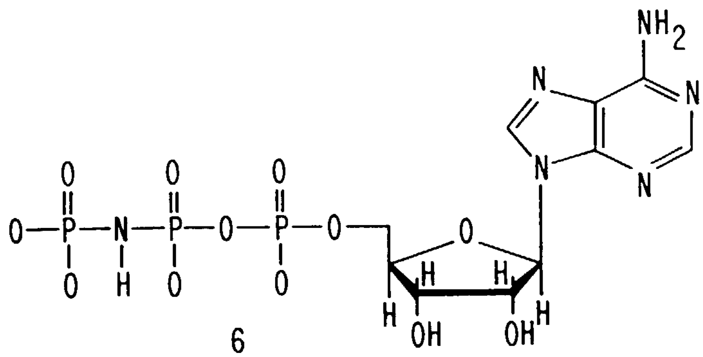


FIG. 15A



5



6

FIG. 15B

FIG. 15C

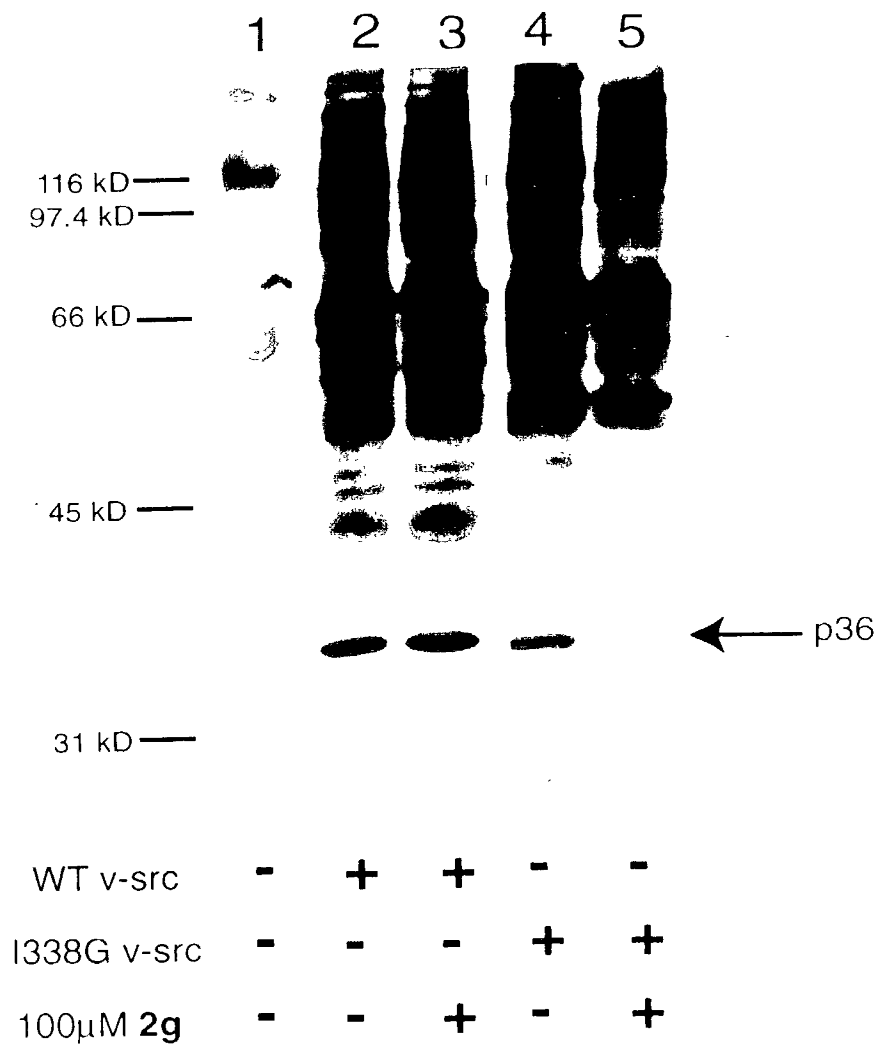

1
2
3
4
5
116 kD
97.4 kD
66 kD
45 kD
p36
31 kD
WT v-src
I338G v-src
100μM 2g
-
+
+
-
-
-
-
-
+
+
-
-
+
-
+

FIG. 16

FIG. 17A

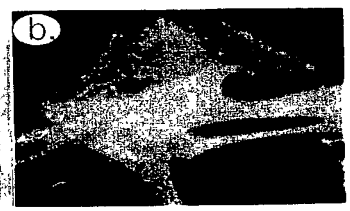


FIG. 17B

Control

100 μM 2g

FIG. 17C

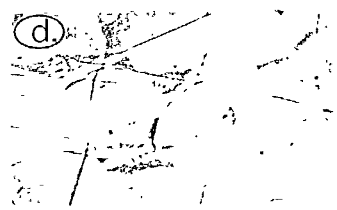


FIG. 17D

FIG. 17E

FIG. 17F

FIG. 17G

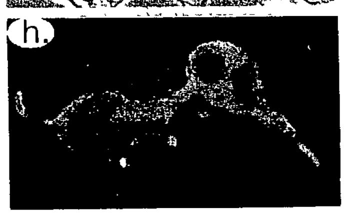


FIG. 17H

FIG. 17I

FIG. 17J

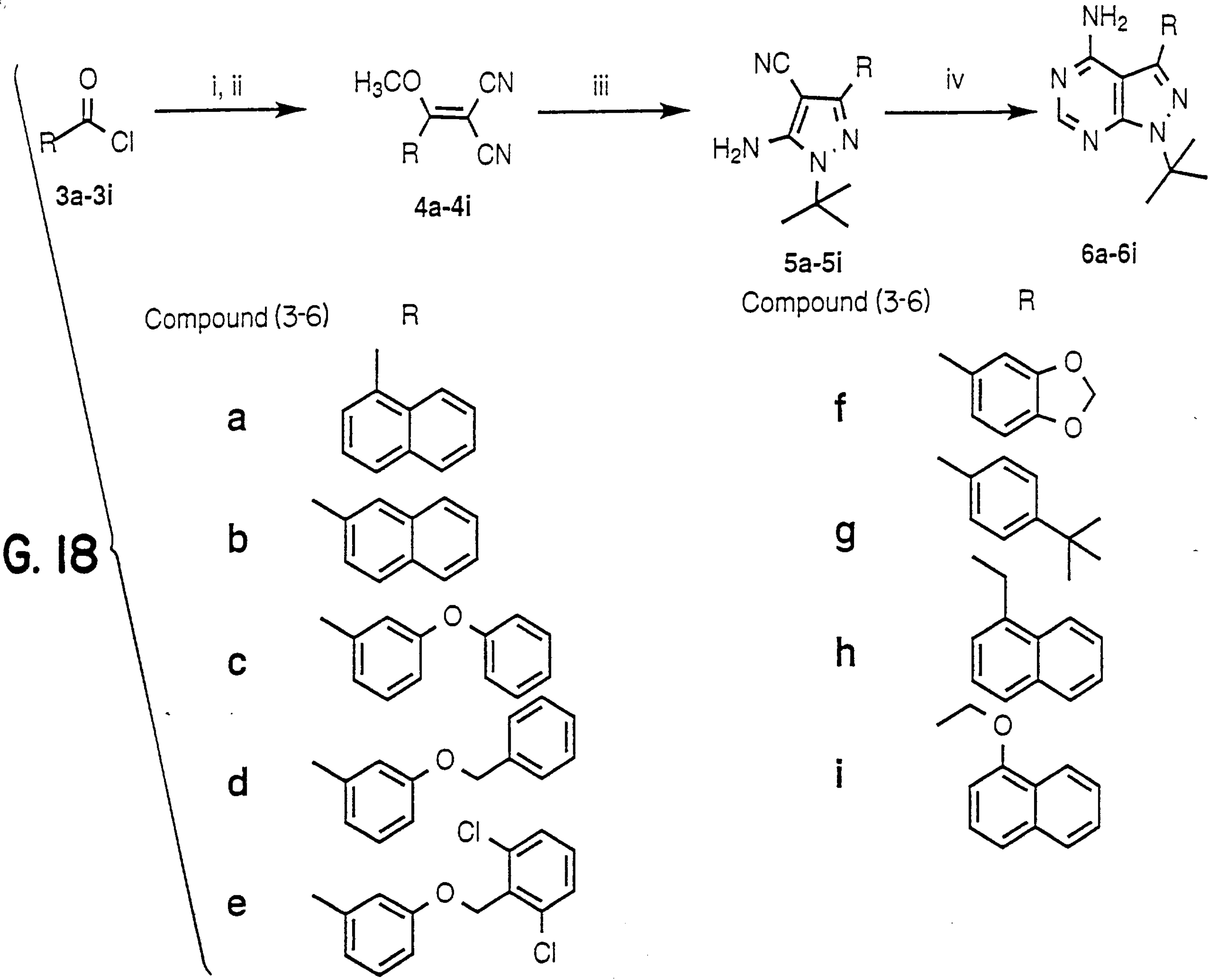


FIG. 18

FIG. 20A

FIG. 20B

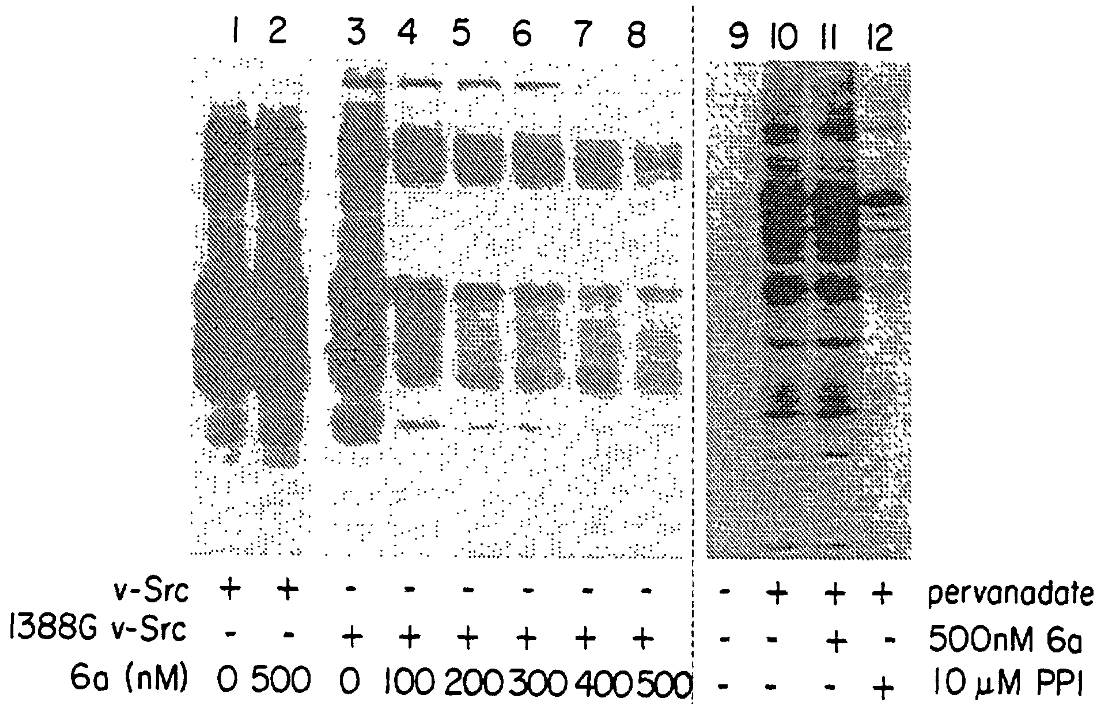


FIG. 21A

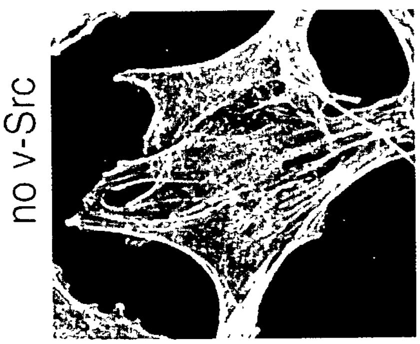


FIG. 21B

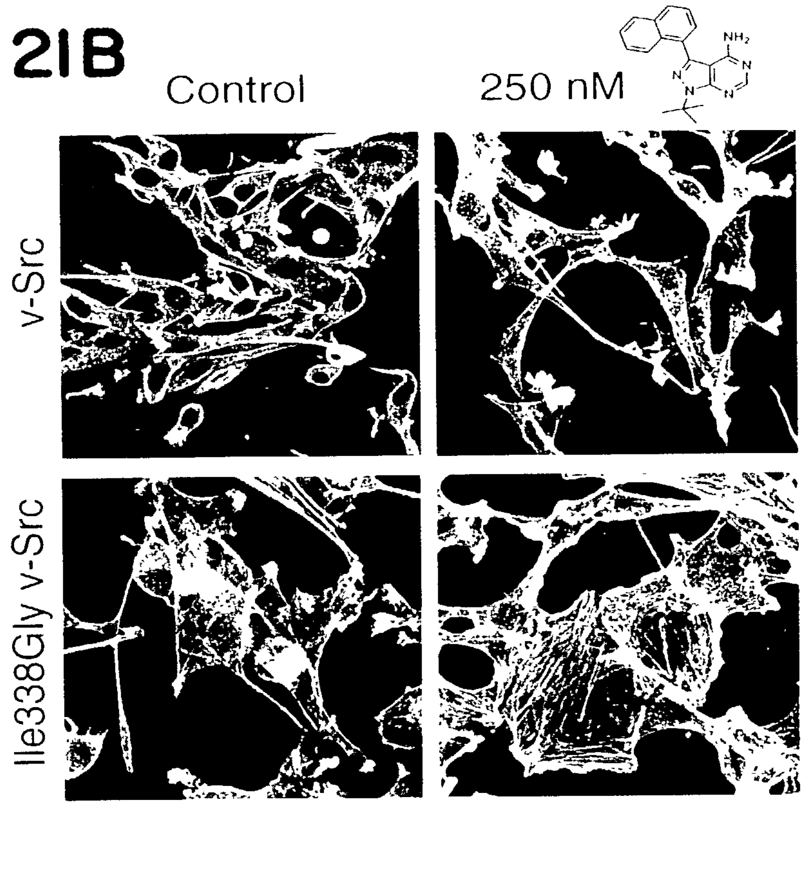
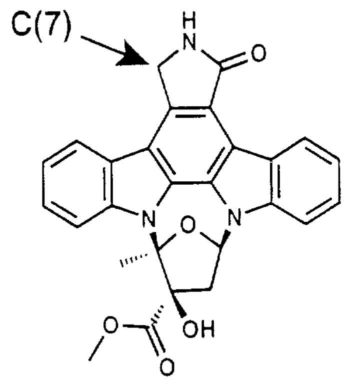
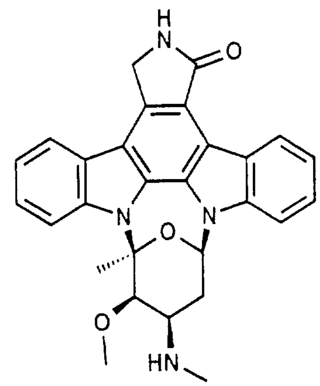


FIG. 22A



(+)-K252a (**1**)



(+)-Staurosporine (**2**)

FIG. 22B

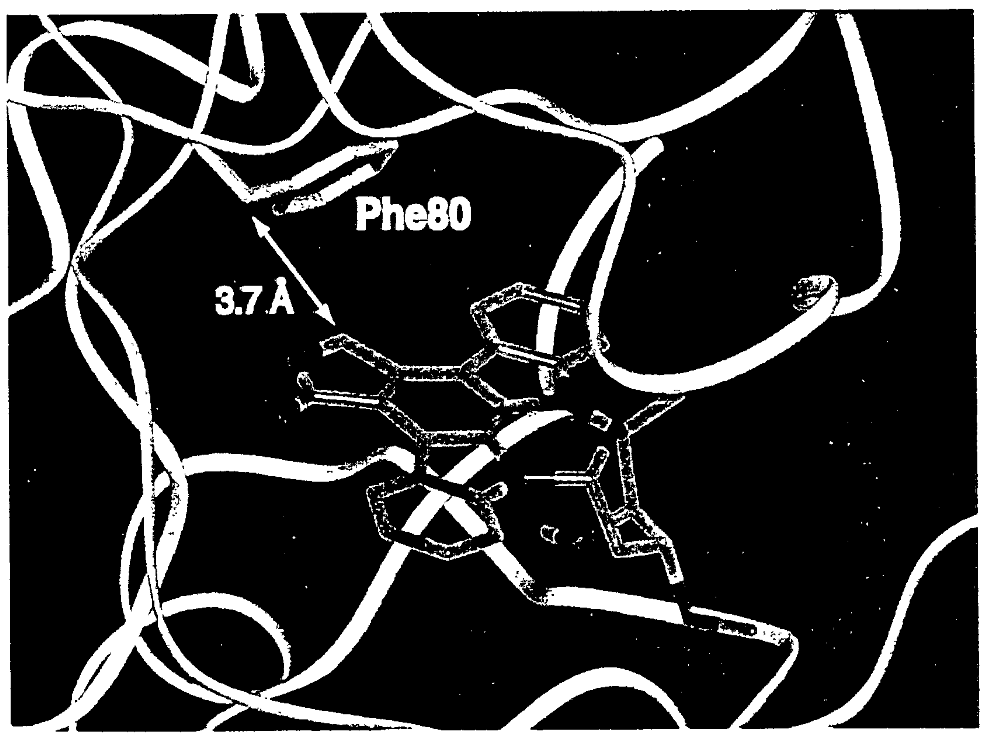
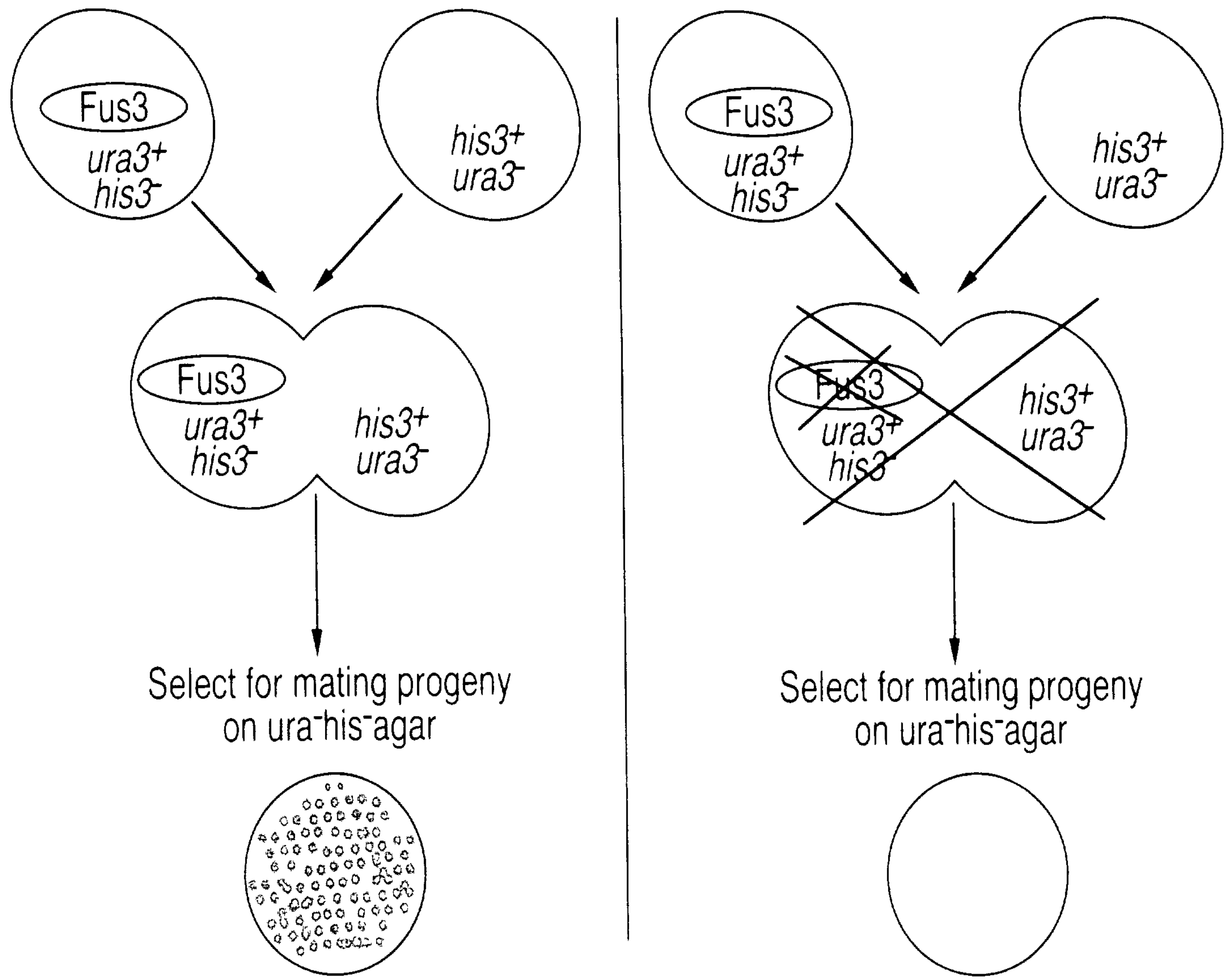


FIG. 23A



Fus3
ura3+
his3-
his3+
ura3-
Fus3
ura3+
his3-
his3+
ura3-
Select for mating progeny
on ura-his-agar
Fus3
ura3+
his3-
his3+
ura3-
Fus3
ura3+
his3-
his3+
ura3-
Select for mating progeny
on ura-his-agar

FIG. 23B

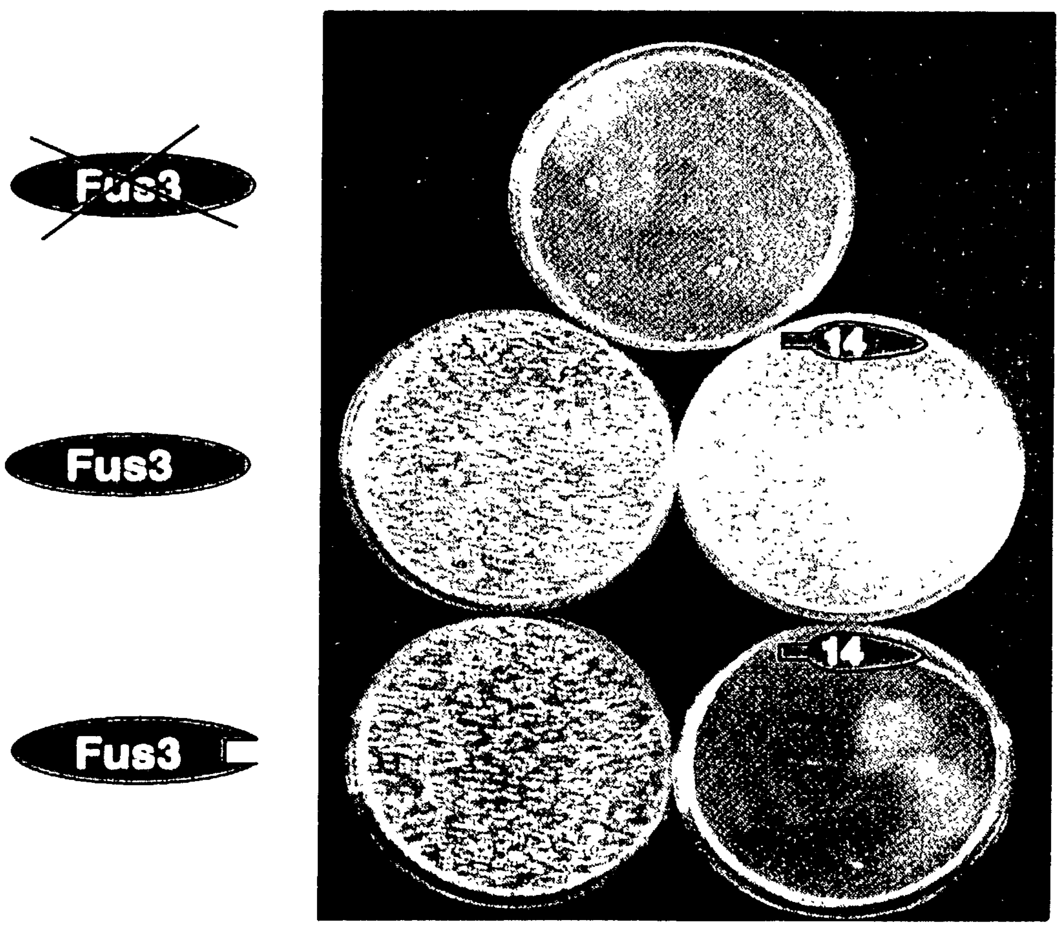


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FIG. 23C

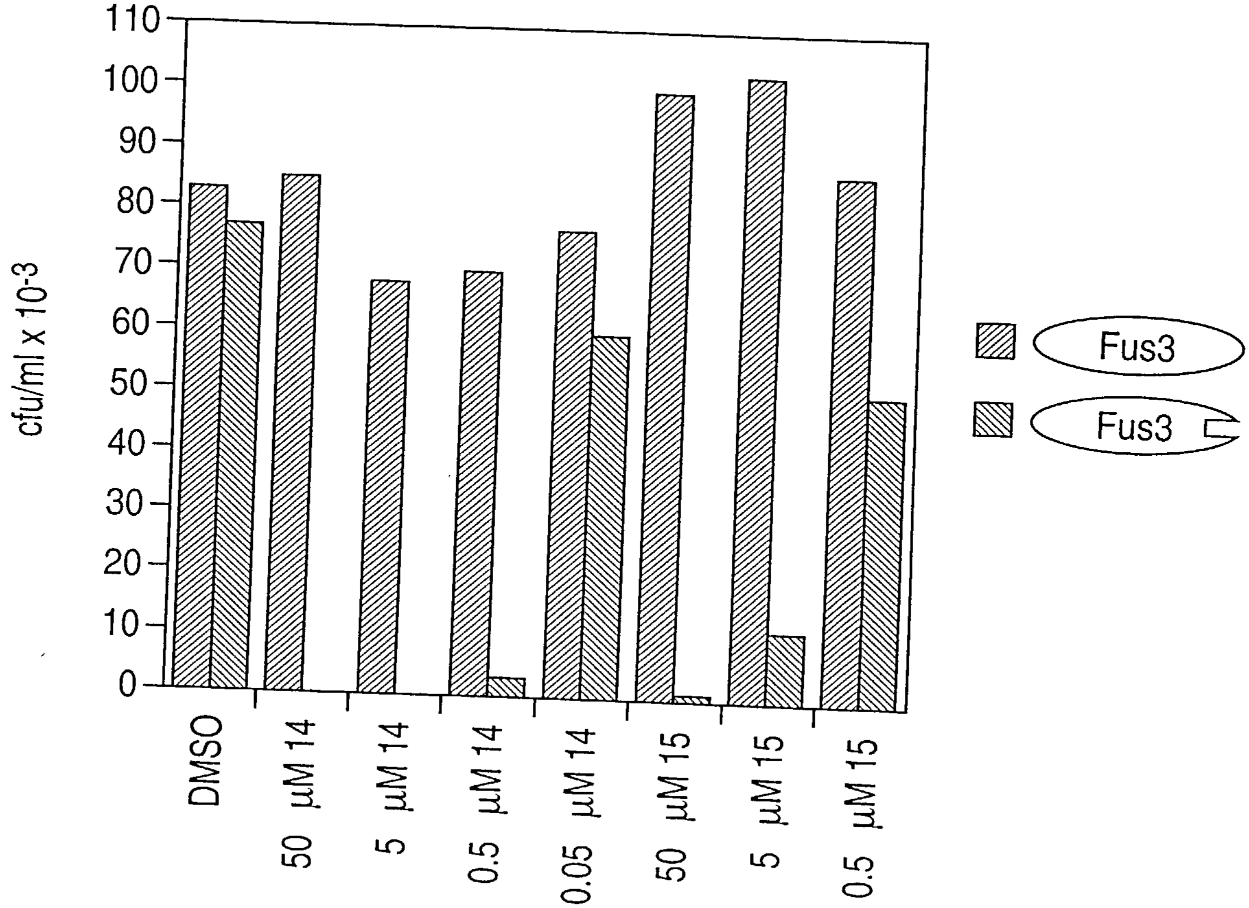
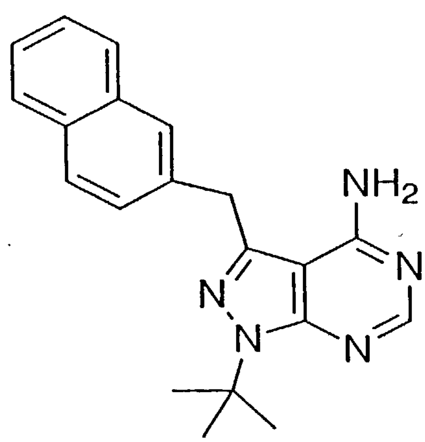


FIG. 24



6j

FIG. 25A

All *S. cerevisiae* genes (6,200)

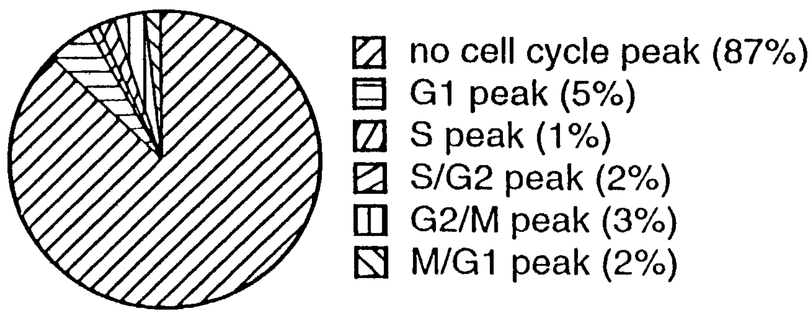
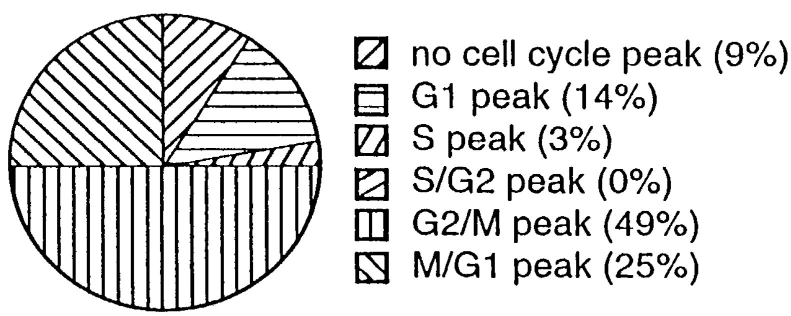


FIG. 25B

Decreases - 120 min (66)



Unreg

AMI1	-3
BAR1	-5.1
PUT4	-3.2
SUN4	-4.1
YBR077C	-2.6
YER067W	-5.5

G1

CTS1	-28.4
GPH1	-2.9
MFA1	-3.2
PRY3	-2.7
RME1	-3.1
RPC10	-41.2
SCW11	-16.4
YER124C	-9.8
YHR218W	-3

S

HTB2	-4.6
MET14	-2.9

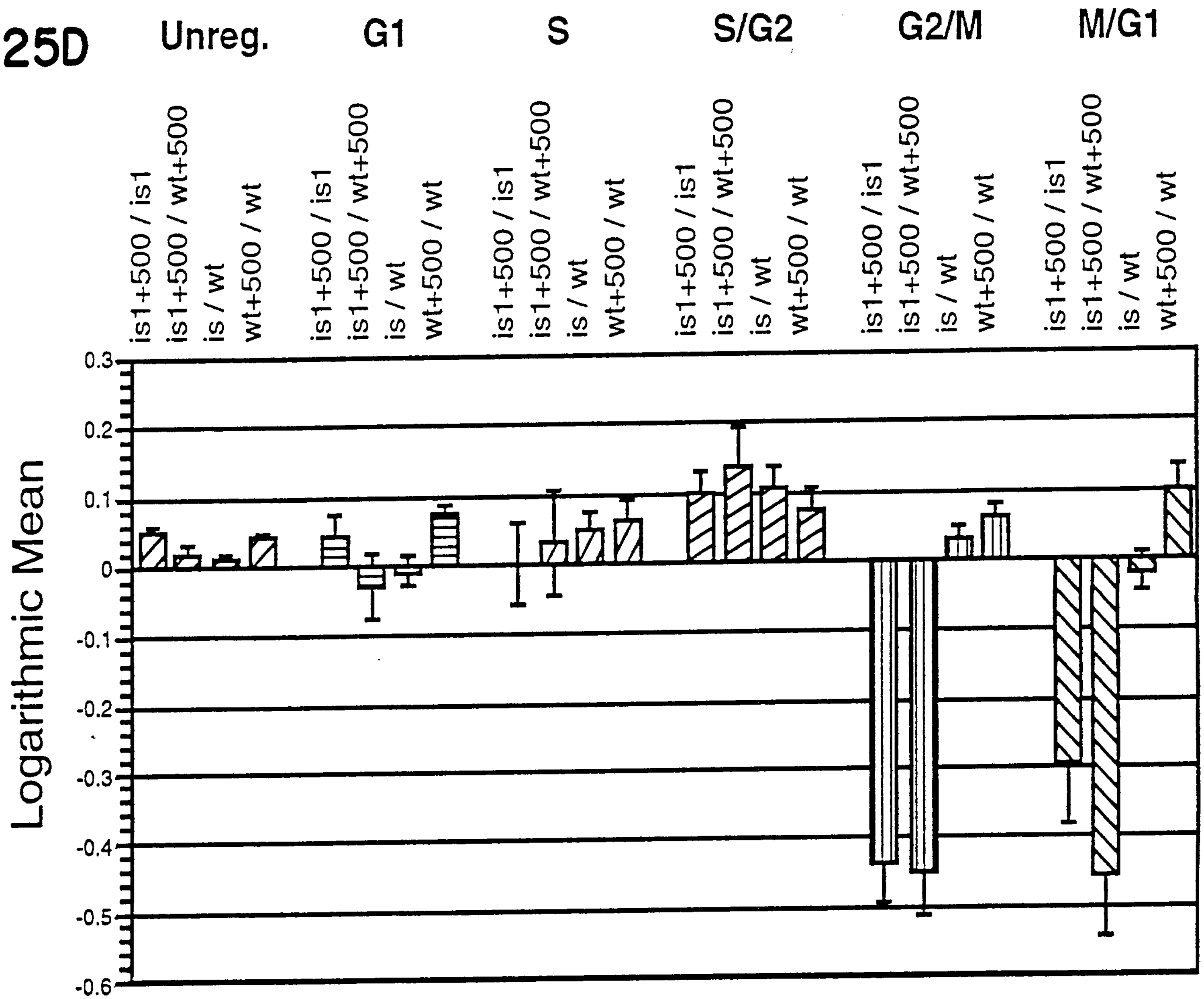
G2/M

ALK1	-3.5
ATF2	-5.1
BNS1	-3.7
CDC20	-4.1
CDC5	-3
CLB2	-4.1
DBF2	-2.6
FAR1	-20.4
HST3	-4.1
MFA2	-6.9
MYO1	-3
PHO11	-4.9
PHO12	-5.9
PHO3	-21.5
PHO5	-10.6
PRY1	-3.2
RPI1	-2.7
SDL1	-3.8
SKN1	-2.5
STE2	-2.5
STE6	-5.8
SUR7	-2.5
SWI5	-3.1
UTH1	-2.5
WSC4	-6.9
YDR033W	-13.6
YIL158W	-3.1
YJL051W	-4
YLR254C	-4.2
YML119W	-4.1
YNL058C	-3.1
YRO2	-7.8

M/G1

AGA2	-6.5
EGT2	-28.3
FAA3	-4.7
GYP6	-2.5
IAH2	-3.4
ICS4	-4.7
MCM3	-2.6
PCL9	-4.9
PIR1	-3.7
PTS1	-3.5
SPI1	-2.6
YGP1	-5.5
YNL046W	-5.7
YNR067C	-19.4
YOR066W	-3.7
YOR264W	-4.7
YPL158C	-4.6

FIG. 25D



Unreg.
G1
S
S/G2
G2/M
M/G1
is1+500 / is1
is1+500 / wt+500
is / wt
wt+500 / wt
Logarithmic Mean
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6

FIG. 26A

Protein Kinase	Kinase Family	Specificity	Cellular Function
v-Src	Src	Tyr	oncogenic transformation
c-Fyn	Src	Tyr	lymphocyte activation
c-Abl	Abl	Tyr	F-actin binding, transcription
CAMK II	calcium/calmodulin dependent	Ser/Thr	long-term potentiation, memory
CDK2	cyclin dependent	Ser/Thr	mammalian cell cycle progression
CDC28	cyclin dependent	Ser/Thr	*S. cerevisiae* cell cycle progression
Fus3	mitogen-activated	Ser/Thr	*S. cerevisiae* mating

FIG. 26B

```
                                                  338
                                                   ▼
v-Src     (318)RHEKLVQLYAMVSE----------EPIYIVIEYMSK--GSLLDFLKGEMGKY
Fyn       (319)KHDKLVQLYAVVSE----------EPIYIVTEYMNK--GSLLDFLKDGEGRA
Abl       (294)KHPNLVQLLGVCTRE---------PPFYIITEFMTY--GNLLDYLRECNRQE
CamK II   (68) KHPNIVRLHDSISEE---------GHHYLIFDLVTG--GELFEDIVAREY
Cdk2      (59) NHPNIVKLLDVIHTE---------NKLYLVFEFLHQ---DLKKFMDASALTG
Cdc28     (66) KDDNIVRLYDIVHSDA--------HKLYLVFEFLDL---DLKRYMEGIPKDQP
Fus3      (67) KHENIITIFNIQRPDSFENF----NEVYIIQELMQT---DLHRVISTQM
```

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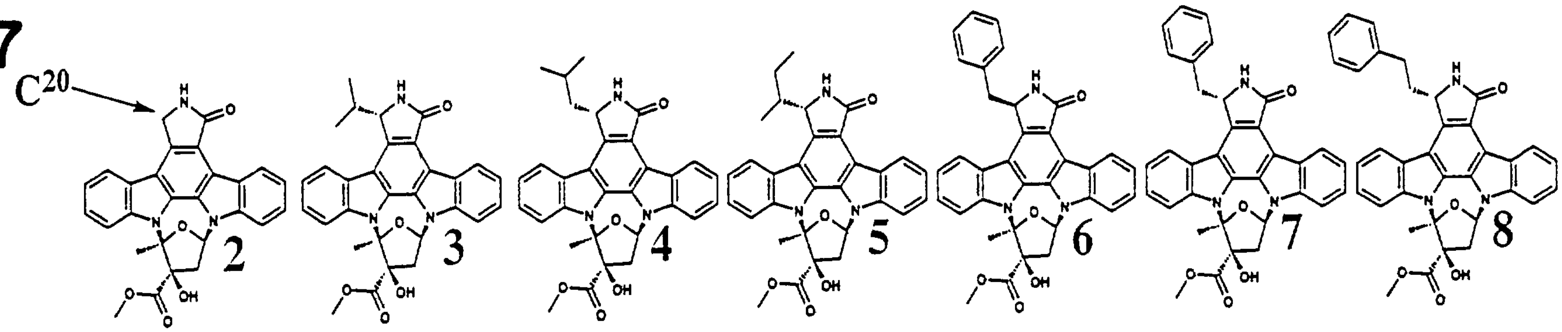
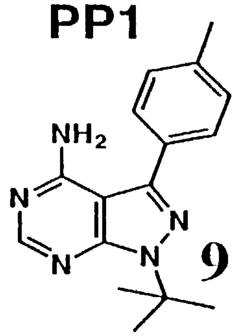
FIG. 27

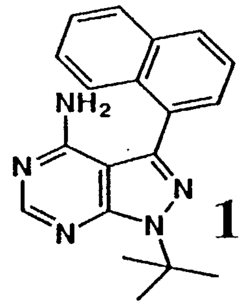


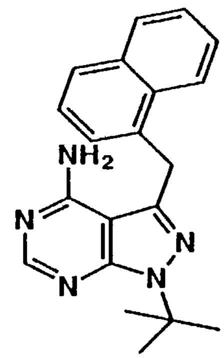
	2	3	4	5	6	7	8
Wild Type							
v-Src	0.020	>33	>33	26	11	3.6	12
c-Fyn	0.17	>33	>33	>33	>33	30	>33
c-Abl	2.2	>33	>33	>33	>33	>33	>33
CDK2	0.020	29	22	5.8	10	1.0	3.7
CAMK II	0.054	2.7	1.7	0.82	0.49	0.13	0.54
Engineered							
v-Src		0.044	0.00023	0.092	0.019	0.0012	0.0021
c-Fyn		0.12	0.00055	0.30	0.057	0.0018	0.0043
c-Abl		>11	3.1	>11	>11	>11	9.6
CDK2		1.9	0.070	>3.7	10	0.15	0.037
CAMK II		2.3	0.95	>3.7	>1.2	0.39	0.0040

FIG. 28

PP1

 9

 10

 11

Wild Type

	9	10	11
v-Src	2.2	1.0	28
c-Fyn	0.050	0.60	1.0
c-Abl	0.30	0.60	3.4
CDK2	22	18	29
CAMK II	17	22	24

Engineered

	9	10	11
v-Src		0.0015	0.0043
c-Fyn		0.0065	0.0032
c-Abl		0.0070	0.12
CDK2		0.015	0.0050
CAMK II		0.097	0.0080